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## HYBRIDISM AND THE RATE OF EVOLUTION IN ANGIOSPERMS

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IN responding to an invitation to contribute to the morning program of the American Society of Naturalists, it has seemed to me that a statement emphasizing some of the morphological features of the greatest of all biological problems, the *modus operandi* of the process of evolution, would be of interest to my fellow biologists. The most distinguished as well as the most profound investigator, which our science has yet produced, Charles Darwin, has unequivocally expressed the opinion in the "Origin of Species," that morphology is the soul of natural history. As I am addressing a body of men who call themselves naturalists, my theme will, I hope, not appear unimportant.

The rate of evolution has not been the same at all periods of our earth's history. There is an agreement among those whose knowledge of the vegetable population of earlier eras makes their opinion worthy of serious regard, that the plant kingdom in former times was in a much less rapid condition of evolutionary change than in the present age. Within the limits necessarily assigned to me it is impossible to state all the probable causes of this notable acceleration in the rate of change in plants. I shall touch only upon two aspects of this problem and of these I shall be able to develop but one.

Extremely important factors in the evolution of plants

have unquestionably been the progressive cooling of our earth's surface, as well as those recently recognized secular periodic twilights of the sun god, known as glacial periods. The latter have worked in an exterminating manner and have wiped out well nigh completely whole types of plants and have left the way clear for the unrestricted development of better adapted forms. For example, at the end of the Paleozoic, in the late Permian, we find world-wide evidence of glaciation, which resulted in the virtual extinction of the great cryptogamic forests, which contributed the raw materials of our most abundant coals. With the passing of the arboreal Cryptogams, the Gymnosperms became the predominant element of the world forests in the Mesozoic. At the end of the Cretaceous there was another age of extinction, which wiped out the mass of Gymnosperms and particularly the Conifers. The naked seeded plants, which prevailed in the medieval period of our earth's history, have in the vegetation of to-day been reduced in the number of species to the merest fraction of seed-producing plants; which in the present age are overwhelmingly angiospermous.

From the present standpoint, however, the progressive but not spasmodic cooling of our earth is of even greater importance. Investigations initiated in my laboratories have made it clear that herbaceous Angiosperms have been derived from woody ones as a response to the increasing coldness of terrestrial climates. Plants of this organization are of such efficiency that they are able to go from seed to seed in a few weeks and thus pass through the inclement winter season in a resting stage. The original researches in this direction were undertaken by Professor Eames. The theme in the past two years has undergone a profitable exploitation by other former students in both botanical and geological publications. The origin of the herbaceous type in the Angiosperms has in itself added a notable impetus to the rate of evolution in the group. Whatever hypothesis one adopts as to the mode of the origin of species, it is quite clear that the

multiplication of generations as well as of individuals, rendered possible by the appearance of the herbaceous type of small size and short reproductive cycle, will contribute to the acceleration of evolutionary processes.

A noteworthy feature, which distinguishes the huge aggregation of Angiosperms now inhabiting the surface of the globe (in the neighborhood of one hundred and forty thousand species) from the saved remnant of the Gymnosperms, is their inherent variability. This high degree of variability has naturally made the Angiosperms a very difficult group from the systematic standpoint and has likewise put them in the foreground in connection with discussions as to the origin of species. Two of the oldest tribes of the coniferous Gymnosperms are the pines and the araucarians. I have had the good fortune to be able to make a careful comparison of structure extending to all important details, between living representatives of these tribes and their predecessors in the Cretaceous of the eastern United States. It is quite clear from these studies that the genus *Pinus* and the genus *Araucaria* in the remote times of the Age of Chalk, differed only in the smallest particulars from their living descendants. The conclusion inevitably follows that the course of evolution here has been very slow. The actual situation corresponds accurately with the data derived from the past. A white pine, compared with an evening primrose or a rose, is relatively constant and invariable.

The remarkable variability of the Angiosperms, as frequently expressed in terms of the difficulty of systematic identification, brings us naturally to the much debated question of the origin of variability. Darwin, as is well known, simply accepted this phenomenon as a fact and did not, after the first, at any rate, attempt to explain the condition in terms of other phenomena. It is interesting, however, to note that in the beginning he was disposed to accept hybridization as the cause of the variability of species and apparently abandoned this belief only because he could find no evidence for its occurrence on a suffi-

ciently extensive scale. Quite recently the view that heterozygosis is responsible for the mutability of species has again been advanced by Lotsy in an interesting article published in the *Archives Néerlandaises*. This author very definitely takes the position that variability in general is due to hybridization, and that true species (not necessarily those of Linnæus and other systematists) are invariable. With this view I am personally in agreement, with the limitation that the statement goes much too far.

It is one of the commonplaces of breeding that the offspring resulting from hybridization is extremely variable and may be characterized by a greater or less degree of sterility. Taking the particular case of the Angiosperms, it is found that when species of lilies, irises, honeysuckles, etc., are crossed, the result is a highly mutable progeny with a greater or less degree of sexual sterility, the latter condition most easily recognized in the microspores or pollen. The main purpose of the present statement is to make it clear to my fellow naturalists that in nature a high degree of variability often exists in the case of the Angiosperms, expressed either in terms of difficulty of systematic determination in view of intergrading forms, or often in the less obtrusive form of multiplication of species in a given genus. This extreme degree of variability is very largely accompanied by the highly significant phenomenon of pollen sterility.

A family of Angiosperms much in the foreground in recent years is the Onagraceæ or Evening Primrose family. In the case of the genus *Enothera* remarkable conditions have been discovered by De Vries. The plants of *O. lamarckiana*, when grown in large numbers, show a number of individuals, sometimes as high as one twentieth of the total number, markedly different in character from typical *O. lamarckiana*. This phenomenon was at first thought to be peculiar to this species of *Enothera* and a great deal of importance was consequently attached to clearing up its somewhat dubious systematic position.



Fortunately we are relieved from the uncertainties necessarily connected with this kind of investigation, by the discovery in more recent years that other and perhaps all species of the genus possess the same features. The activity of systematic botanists in recent years in making new species of *Oenothera* is highly significant in the present connection. The exceptional individuals which grow up in cultures of species of *Oenothera* have been termed by De Vries and his disciples "elementary species." The biological world has been asked to believe that in the appearance of these new forms in cultures of *Oenothera*, we have the phenomenon of mutation or the origin of species at a leap. This view of the matter is, however, open to serious question. The species of *Oenothera*, as well as their so-called mutants, are distinguished by a degree of pollen sterility often extreme. This condition has convinced so accomplished a geneticist as Professor Bateson that the so-called elementary species of *Oenothera* are segregates resulting from previous hybridization. This view of the matter is supported by the fact that the products of hybridization are often relatively fixed forms, as indeed has been noted by Brainerd in his extremely interesting observations on hybrid wild violets.

Obviously the question of possible mutation in the genus *Oenothera* entered into a new and biologically more advantageous phase when other species than *O. lamarckiana* came into the discussion. Clearly a still wider view should even more clarify the situation. Two years ago Miss Ruth Holden, who is at present living in Cambridge, England, made the interesting discovery that the common fireweed, *Epilobium angustifolium*, growing wild near Cambridge and also cultivated in the Cambridge botanic garden, was characterized by a large degree of sterility of pollen. She at once generously communicated her discovery to me and at the same time suggested a reason for the condition of pollen found in the English specimens of *Epilobium angustifolium*. I must here remind you that under the genus *Epilobium* are included two distinct

subgenera, namely *Chamænerion*, distinguished, among other features, by its distinct pollen grains; and *Epilobium* proper having its pollen grains in groups of four. *E. angustifolium* belongs to the section *Chamænerion*, and in the southern part of Canada and the Northern States has no allied species except in mountainous regions (*e. g.*, mountainous Quebec and Colorado). Acting on the suggestion supplied by Miss Holden's discovery, Mr. C. A.

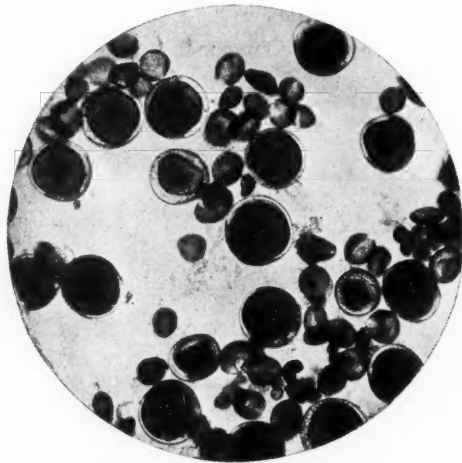


FIG. 1. Pollen of hybrid *Iris germanica*.

Forsaithe, one of my graduate students, has investigated the conditions of sterility found in species of *Epilobium* belonging to the section of *Chamænerion*. Through the kindness of the Gray Herbarium of Harvard University he has been able to study some two hundred specimens from various geographic regions. The conditions in *Epilobium* (*Chamænerion*) *angustifolium* in the northern part of its range, where it coincides in distribution with its allied species, *E. latifolium*, are most interesting. Nearly nine tenths of the specimens showed the pollen to be imperfect. In contrast, the material from the southern limits, where *E. angustifolium* does not coincide in distri-

bution with *E. latifolium*, are almost uniformly characterized by a high degree of perfection. To be specific, specimens from Ontario, western Quebec, and New Hampshire and Massachusetts show pollen perfectly developed or at most with a few grains disorganized. Mr. Forsaith extended his investigation, again through the courtesy of the Gray Herbarium, to the other genera and species of the Onagraceæ, with similar results. The investigation as a whole will be described elsewhere, but it will be necessary



FIG. 2. Pollen of *Zauschneria californica*, a monotypic representative of the Onagraceæ.

to consider a few more illustrations in the present connection. There is one quite monotypic species in the order, namely *Zauschneria*. It was found that in this the pollen is practically perfect and the same state of affairs is present in the two geographically limited species of *Gongylocarpus*, one occurring in Vera Cruz and the other on the opposite side of the continent in Lower California. The general situation in the case of the Onagraceæ, a family much in the foreground at the present time by reason of the investigations of De Vries and his disciples, is that monotypic species or those geographically isolated have

perfect pollen and are little characterized by variability; while where the species are numerous and coincident in their range both variability and pollen sterility are conspicuous.

We may now consider another highly variable group, which has not infrequently been called a hybrid family, namely the Rosaceæ. The genera *Rosa*, *Rubus* and *Crataegus* are notable for the extreme difficulty they have offered from the systematic point of view. Three of my

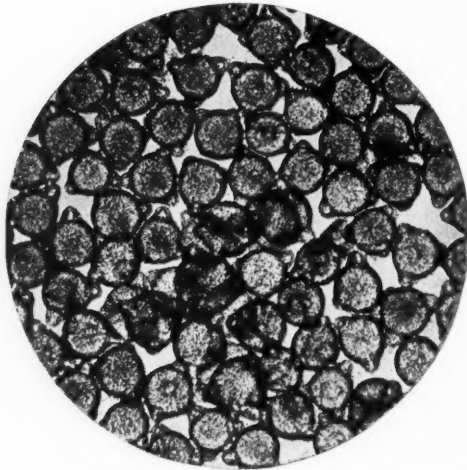


FIG. 3. Pollen of *Chamaenerion* (*Epilobium*) *angustifolium* from Massachusetts.

graduate students have investigated these genera and the

results may be conveniently summarized by reference to the genus *Rubus*. In the case of *Rubus*, in regions where it has been exhaustively studied, there is almost no end to the species which may be set up. In Europe, in fact, the species have mounted into the thousands. The situation may for the sake of brevity be considered under three heads. First, there are species which range together and have flowering periods which overlap—a condition common to the mass of our ordinary *Rubi*. In *Rubus villosus*,

the blackbriar, and *R. strigosus*, the wild red raspberry, both very variable species, the pollen is extremely bad. Where these species occur on islands, however, the pollen is generally much more perfect, probably as the result of isolation. I have noticed, for example, that *R. villosus* and *R. strigosus* from Cape Breton Island have considerably better pollen than that found in the case of continental material of the same two species. What is true of these particular species holds more or less well for a



FIG. 4. Pollen from *Chamaenerion (Epilobium) angustifolium* from the vicinity of Cambridge, England, showing abortive grains.

large number of others of similar range and flowering periods. Next may be considered a species of limited geographic range, namely *R. deliciosus* from the Rocky Mountains. Here the pollen is practically entirely perfect, a few defective elements being occasionally found. Last may be described *R. odoratus*, the so-called flowering raspberry, which blossoms after the mass of other species have shed their flowers. Here, as one might expect, the pollen is highly perfect and practically unmingled with shrivelled grains. A general study of the Rosacæ, which can not even be summarized in the brief

time at my disposal, shows clearly that propinquity, geographical or phenological, is to a large extent correlated with pollen imperfection in the group.

Limitations of time make it necessary to proceed summarily with other illustrations. Next may be cited the Betulaceæ and Fagaceæ. Each of these orders has one strikingly polytypic species, *Betula* in the one case, and *Quercus* in the other. Interestingly enough, it is in these two genera that variability and gametic sterility coincide.



FIG. 5. Pollen of *Rubus deliciosus* from the Rocky Mountains, showing well developed grains.

One might continue at length through the Dicotyledons, but one other example must suffice for this division of the Angiosperms. The Solanaceæ have one huge genus, *Solanum* itself, in which there are nine hundred species. In this genus not only is there extreme variability, but also a large degree of pollen sterility. In the monocotyledonous division we may start with the grasses. Monotypic grasses have perfect pollen, as is illustrated, for example, by the wild rice, *Zizania aquatica*. In the genus *Alopecurus*, with numerous and propinquitous species, on the contrary the pollen conditions frequently indicate gen-

etical contamination. Proceeding to aquatics, in the Potamogetonaceæ, the monotypic *Zannichellia* and *Zostera* have perfectly developed microspores; while *Potamogeton*, with its numerous species, is often distinguished by a large degree of pollen imperfection. Similar statements hold in a like sense in regard to members of the Alismaceæ, Sparganiaceæ, etc.

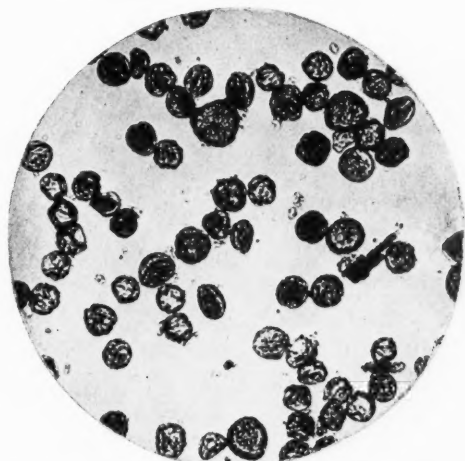


FIG. 6. Pollen of *Rubus villosus* (Blackbriar), showing high degree of imperfection.

The pressure of time compels a summing up of the situation without further references to detailed facts, which will be supplied by publications soon to appear. The general condition in the Angiosperms in contrast to the Gymnosperms is a large degree of variability in the species. Where the species are highly inconstant and cause great difficulty to the systematist, as, for example, in the Onagraceæ, Rosaceæ, Solanaceæ, Birches, Oaks, etc., there is often a large degree of pollen sterility. Where isolation, geographical, phenological or specific, is present the contents of the anther sacs are strikingly perfect in their development. In other words, where interspecific crossing is possible, there is often clear evidence of its presence



in the form of a high degree of variability, accompanying a considerable manifestation of sterility in the gametic cells, particularly the pollen. In the numerous species of *Rosa* or *Oenothera*, we find in regard to both variability and the phenomenon of sterility, a marked contrast to the also numerous species of the very old genus *Pinus*. In *Pinus* there is practically no imperfection in the develop-

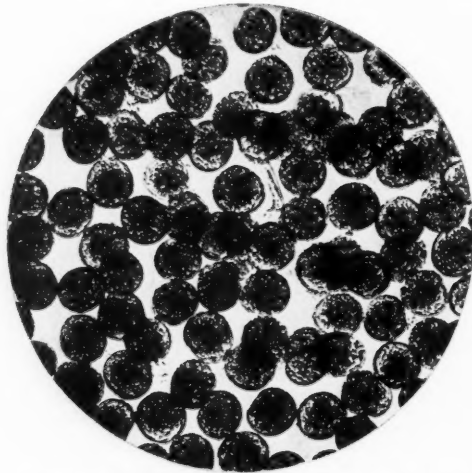


FIG. 7. Pollen of *Zizania aquatica*, a genus with few isolated species.

ment of the microspores, even in exotic species, and the species are very clearly marked and constant.

If associated variability and gametic sterility are reliable indications of hybridization, then it becomes clear that the Angiosperms, unlike the Gymnosperms and the mass of the vascular Cryptogams, are often characterized by heterozygosis. It has been recently suggested that pollen imperfection is not so much an evidence of hybridization as of mutability. This criticism appears to fail for various reasons. First, for nearly a hundred years practically all students of hybridization in plants have noted pollen sterility and imperfect development of the seed as peculiar characteristics of hybrids. Secondly,

in genera with often highly sterile species, such as *Rubus*, the species which are isolated for any reason from the rest have either perfect pollen or manifest a much less marked degree of sterility. An objection urged by De Vries to gametic degeneracy as a criterion of hybridism needs apparently only to be stated to supply its own refutation. The distinguished plant physiologist of Amsterdam, in a recent article in which he criticizes the writer's

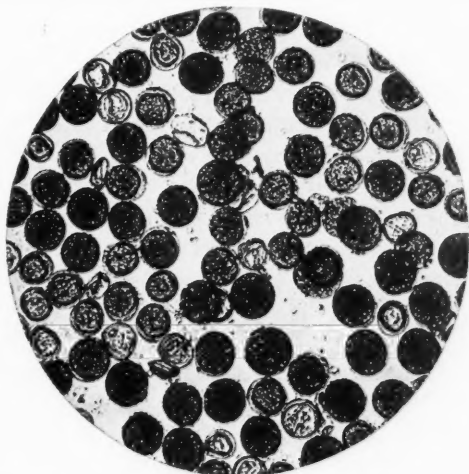


FIG. 8. Pollen of *Alopecurus pratensis*, showing high degree of imperfection which may occur in a polytypic genus.

attitude in regard to the intimate relation between defective pollen, hybridization and so-called mutation, somewhat superciliously, states that the degeneracy of spores in connection with the development of the megaspores of the heterosporous vascular Cryptogams (and one might add the seed plants as well) might with equal validity be regarded as evidence of hybridism in the megasporic sporangia. One has only to carry De Vries's argument to its logical conclusion to prove its entire fallacy. Since microsporic sporangia (in which there is no spore degeneration apart from hybridism) and megasporic sporangia occur ordinarily or at least primitively on the same plant,

it follows that so far as the phenomenon of spore degeneration is concerned some sporangia (the megasporangia or seeds) are of hybrid origin and others (the microsporangia or anthers) are not. The logical absurdity of this conclusion will be clear to every one.

There seems to be no question on the basis of the well-established criteria of hybridism, that many Angiosperms present clear indications that their species are of heterozygous origin. Since one of the most efficient methods of inducing variability in connection with the development of improved varieties of plants is hybridization, often on a very large scale, it seems not unreasonable to regard spontaneous hybridization in the Angiosperms (the evidences for which are so numerous and so impressive) as having an incalculably large effect on their rate of evolution. There is, however, apparently no reason for assuming a similar condition in the Gymnosperms and the vascular Cryptogams. The great and indeed overwhelming advantage which the Angiosperms have secured in the struggle for existence over the lower groups of vascular plants is apparently connected in an intimate way with hybridism on the one hand and the development of herbaceous types (in response to progressive climatic refrigeration) on the other. If this conclusion is correct we must reject the assumption of universal hybridism as the sole cause of variation put forward by Lotsy as much too sweeping. Small variations unquestionably characterize the Gymnosperms, and in the course of long geological time have availed in the absence of competition from heterozygous types, with a much greater range of variability and consequently a higher potentiality of evolution. It is obviously impossible for the homozygous Conifers to make headway against the characteristically heterozygous Angiosperms. The small variations of homozygous stocks clearly prevailed in the earlier history of our earth, while the more rapid changes which have ensued in later times are correlated, so far as plants are concerned, at any rate, with marked physiographic and

climatic differentiation, and most important of all with the phenomenon of heterozygosis.

In conclusion the situation may be summarized. The phenomenon of variation in the older types of plants is still unexplained and must apparently be accepted as an ultimate characteristic of living matter. In the case of those groups of plants, which have achieved predominance under the present climatic conditions of our earth, hybridism has clearly played a large rôle in the acceleration of the processes of evolution. The peculiar conditions presented by the species of *Oenothera*, which have been put forward by De Vries in favor of his mutation hypothesis, are obviously only a particular case of the manifestation of the natural hybridism, which is so widespread a feature of the Angiosperms. The mutation hypothesis has suffered a process of rapid disintegration of late and it is increasingly clear on the botanical side that where the term mutation is used it ordinarily indicates changes which are the result of previous hybridization. Concerning the Animal Kingdom the trend of opinion is apparently setting equally strongly against mutation. My zoological colleague, Professor Castle, has recently declared himself in no uncertain terms against the hypothesis of mutation, an expression of opinion not the less convincing because he originally held the view that mutation was a necessary pendant to Mendelism. He is now able to explain to himself the appearance of new characters as a result of the summation of small variations, which is essentially the Darwinian position.

A FURTHER ANALYSIS OF THE HEREDITARY  
TRANSMISSION OF DEGENERACY AND  
DEFORMITIES BY THE DESCENDANTS  
OF ALCOHOLIZED MAMMALS. II

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THE INFLUENCE OF INTERNAL AND EXTERNAL FACTORS ON  
THE QUALITY OF THE OFFSPRING

Table II gives the relationship between the size of the litters and the mortality of the descendants from different combinations. It brings out in a way the variable internal and external factors to be considered in interpreting the conditions of the members of the numerous litters of animals. The external factor considered in the table is one of nutrition or environment, depending upon the number of young developed in the uterus at any one time. The table indicates the influence of an internal factor, the germ plasms concerned in mating related or non-related animals. Four combinations are considered: pairs of normal non-relatives, pairs of alcoholic non-relatives, pairs of normal relatives, and pairs of alcoholic relatives.

The first vertical column shows that in mating together normal non-related guinea pigs of the stocks used in these experiments the average litter contains 1.96 individuals. Fifty-one and eleven hundredths per cent. of the young were found in litters of two, and 20 per cent. of the animals occurred in litters of three. Fifteen and fifty-five hundredths per cent. of the animals were born in litters of only one young, and 13.33 per cent. in litters of four individuals.

The next space below in the table shows the number and percentage of individuals living over three months

TABLE II

THE SIZE OF LITTERS AND MORTALITY OF DESCENDANTS FROM DIFFERENT COMBINATIONS

	Normal Lines 90						Alcoholic Lines 401						Normal Inbred 30						Alcoholic Inbred 135					
	1 in litt. 14	2 in litt. 46	3 in litt. 18	4 in litt. 12	1 in litt. 84	2 in litt. 214	3 in litt. 87	4 in litt. 16	1 in litt. 12	2 in litt. 18	3 in litt. 0	4 in litt. 0	1 in litt. 12	2 in litt. 60	3 in litt. 0	4 in litt. 0	1 in litt. 38	2 in litt. 76	3 in litt. 21	4 in litt. 0	1 in litt. 1	2 in litt. 2	3 in litt. 3	4 in litt. 4
Total number.	(15.55%)	(51.11%)	(20%)	(13.33%)	(20.94%)	(53.36%)	(21.69%)	(3.99%)	(40%)	(60%)			(40%)	(60%)			(28.14%)	(56.29%)	(15.55%)		(1.62)			
Lived over 3 months....	(Average number of young per litter 1.96)				(Average number of young per litter 1.79)								(Average number of young per litter 1.43)				(Average number of young per litter 1.62)							
Died within 3 months....	1 in litt. 12	2 in litt. 40	3 in litt. 11	4 in litt. 4	1 in litt. 54	2 in litt. 109	3 in litt. 19	4 in litt. 3	1 in litt. 12	2 in litt. 12	3 in litt. 0	4 in litt. 0	1 in litt. 12	2 in litt. 66	3 in litt. 0	4 in litt. 0	1 in litt. 29	2 in litt. 20	3 in litt. 0	4 in litt. 0	1 in litt. 1	2 in litt. 2	3 in litt. 3	4 in litt. 4
	(85.71%)	(86.95%)	(61.11%)	(33.33%)	(64.28%)	(50.43%)	(21.83%)	(18.75%)	(100%)	(66.66%)			(100%)				(76.31%)	(26.31%)			(76.31%)			
	(All together 74.44%)				(All together 46.13%)				(All together 80%)				(All together 80%)				(All together 36.29%)				(All together 36.29%)			
Deformed....	1 in litt. 0	2 in litt. 0	3 in litt. 0	4 in litt. 0	1 in litt. 30	2 in litt. 105	3 in litt. 68	4 in litt. 13	1 in litt. 0	2 in litt. 6	3 in litt. 0	4 in litt. 0	1 in litt. 0	2 in litt. 6	3 in litt. 0	4 in litt. 0	1 in litt. 9	2 in litt. 56	3 in litt. 21	4 in litt. 0	1 in litt. 23	2 in litt. 68	3 in litt. 100	4 in litt. 0
	(14.28%)	(13.04%)	(38.88%)	(66.66%)	(35.71%)	(49.06%)	(78.16%)	(81.24%)	(All together 20%)				(All together 20%)				(23.68%)	(73.68%)	(100%)		(23.68%)			
	(All together 25.56%)				(All together 53.89%)				(All together 20%)				(All together 20%)				(All together 63.70%)				(All together 63.70%)			
Undersize (less than $\frac{2}{3}$ of the average weight)....	1 in litt. 0	2 in litt. 0	3 in litt. 0	4 in litt. 0	1 in litt. 1	2 in litt. 4	3 in litt. 3	4 in litt. 2	1 in litt. 0	2 in litt. 1	3 in litt. 0	4 in litt. 0	1 in litt. 0	2 in litt. 0	3 in litt. 0	4 in litt. 0	1 in litt. 1	2 in litt. 2	3 in litt. 4	4 in litt. 0	1 in litt. 1	2 in litt. 4	3 in litt. 0	4 in litt. 0
	(1.19%)	(1.86%)	(3.44%)	(12.50%)	(1.19%)	(1.86%)	(3.44%)	(12.50%)	(All together 3.33%)				(All together 3.33%)				(2.89%)	(5.26%)			(2.89%)			
	(All together 2.49%)				(All together 5.23%)				(All together 3.33%)				(All together 3.33%)				(All together 14.81%)				(All together 14.81%)			

in the different-size litters. Almost 86 per cent. of the individuals born one in a litter lived, and about 87 per cent., 40 out of 46, of those born two in a litter lived. When there were three in a litter, however, only 61 per cent. lived and of those born four in a litter, it happened that only one third of them survived, though there were only a few in all. Of the total number of young from normal non-related parents 74.44 per cent. lived. Judging from these statistics litters of one or two young are the most vigorous and individuals born in litters of three or four are not so likely to be strong and long-lived.

The next space below gives the mortality records, which, of course, is merely another way of bringing out the above statements. The space following contains the number of deformed animals, but from the normal matings not one such individual has been produced. The last space gives the number of small-size or dwarf specimens also, none of which occur among these litters from normal non-related parents.

The second vertical column contains a similar analysis of the influence of the size of the litter on the mortality and condition of the young born from non-related alcoholic parentage. This not only includes the offspring from directly treated animals, but also other matings of non-relatives belonging to the alcoholic lines. Here again the majority of all the young, 53.36 per cent., are born in litters of two. Litters containing three are next in frequency, followed by litters of only a single individual. Of the total number of offspring produced by alcoholic parents 21.69 per cent. occurred in litters of three, and only about 4 per cent. of the offspring were members of litters of four individuals. The average number of young in the litters from these animals is 1.79, somewhat smaller than from normal matings.

The space below shows that in all only 46.13 per cent. of these young survived, whereas more than half as many more, or 74.44 per cent., from normal parentage lived over three months. The most vigorous animals are those



born only one in a litter. Sixty-four and twenty-eight hundredths per cent. of them lived. While about 51 per cent. of the two-in-a-litter individuals survived, only about 22 per cent. of the young born three in a litter were capable of surviving, and only 18.75 per cent. of the individuals from the litters of four lived more than three months. These figures indicate that the offspring from similarly injured parents are more capable of survival when born in a small litter of one or two than when contained in larger litters of three or four.

This is not on account of the fact that the treated or degenerate mother is more incapable of nourishing the larger litters, since the same is true of the larger litters from normal mothers, as shown by the previous column. The fact is that all young of large litters tend to be small and weak at birth, whereas a single young is far better accommodated. For these reasons it is always of importance to know the size of the litter in which an animal was born in estimating the degenerate qualities it may possess as compared with the qualities of another individual. For example, one animal may appear larger and stronger than another, and yet when bred will give rise to more degenerate offspring than the weaker individual. Although having a vigorous body, its germ-cell complex was not so good as that of the weaker animal, from a larger litter which produces better offspring. Therefore, the small weak males bred to normal females do not always give the poorer results when compared with the matings of stronger males and normal females.

The next space is the reverse of the one above and shows the percentages of mortality among the offspring derived from alcoholic non-relatives. More than half of the young, 53.86 per cent., from these combinations die soon after birth, a mortality record just twice as high as that of the control animals.

The next space shows the frequency of deformities among such young. Here it is again clearly indicated that the animals born one in a litter are better than those



FIG. 1. 521 albino  $F_2$  ♀ (two alcoholic grandmothers, both grandfathers normal). Lived only one day after birth; the meninges of the brain were filled with blood. Gross tremor and complete paralysis of right side. Cataracts, both crystalline lenses being entirely opaque. The photograph shows the outstretched paralyzed right extremities while the left legs are held in a normal position in their effort to support the body. (Birth weight, 54 grams.)

FIG. 2. 506 mouse and yellow  $F_{2.3}$  ♂ (two paternal great-grandmothers and the maternal grandmother alcoholic, slightly inbred). Gross tremor and complete paralysis of left side so unable to walk. Cornea of right eye opaque. Photograph shows the powerless condition of the outstretched left legs with the right legs attempting to support the body. (Birth weight, 57 grams.) The two figures are at different magnifications.

from litters of two, which are in turn better than the members of the litters of three individuals. Only 2.25 per cent. of the 84 individuals born in litters of one were deformed. While 9 of the 214 individuals born two in a litter, or 4.2 per cent., almost twice as many, were deformed. And 10 of the 87 animals born three in a litter were deformed, or about 11.5 per cent., which is almost five times higher than the number of deformities found among the animals born in litters of single individuals. Among the descendants of alcoholic non-relatives there was in all 5.23 per cent. of deformed specimens, whereas not one deformed animal arose from similar normal matings.

The last space of this column indicates the number of dwarf or undersize animals produced in the different litters from non-related alcoholic lines. Among the 84 animals born one in a litter only a single individual was of unusually small size. The 214 animals born in litters of two were all of average size except four, or stated exactly, 1.86 per cent. of them were undersize. In the litters of three 3.44 per cent. of the animals were small, while 12.5 per cent. of the members of litters of four were small specimens. Here again it is shown that the members of large litters are not so uniformly up to the standard of size and vigor as animals born in litters of only one or two individuals.

The third vertical column gives a similar analysis of the few normal inbred individuals which have been produced during the time of the experiment. There are not many such matings, as a general effort has been made to avoid inbreeding the control animals since this might be considered to vitiate the results.

The few young produced by inbred normal matings have all been in litters of only one or two offspring, so that the size of the litters averages only 1.43 individuals. The size of the litters is, therefore, smaller than from either the non-related normal or alcoholic animals. Eighty per cent. of the young have survived, more, however, from the

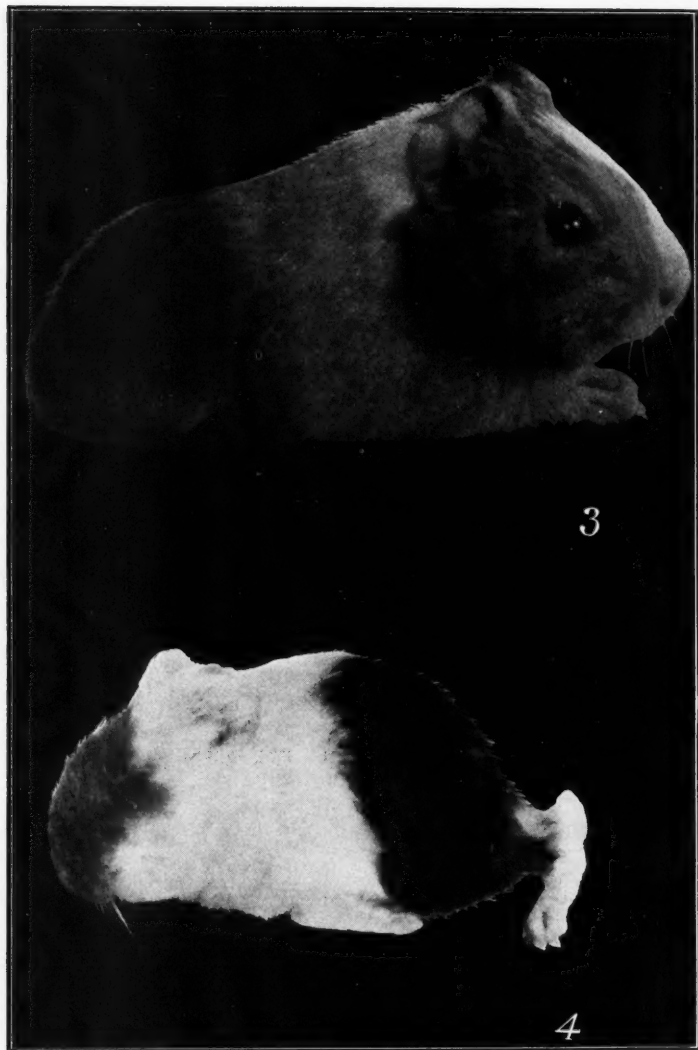


FIG. 3. 701 agouti, yellow and white, normal  $F_3$  ♂. A normal animal from the fourth generation of the control; slightly inbred, natural size. Birth weight, 63 grams.)

FIG. 4. 599 black, white and red.  $F_3$  ♂. A degenerate animal from the fourth generation alcoholic lines; no inbreeding; the paternal grandmother had both parents alcoholic and the maternal grandfather had both parents alcoholic. So there were two alcoholic great-grandmothers and two alcoholic great-grandfathers; the other four great-grandparents were normal and one grandmother and one grandfather had no alcoholism in their ancestry. The parents were ♂ (NN) (AA) ♀ (AA) (NN). The animal lived seven days and died in convulsions. The photograph shows the front limbs bent under the body and the animal is unable to raise the head. It weighed only 35 grams at the time of death, having lost 7 grams. While the above normal animal weighed 63 grams, actually a little small, at birth and all normal animals increase in weight rapidly from that time.

litters of one than from the litters of two. Not one deformed animal has resulted from these normal inbred matings and only one individual of the thirty was less than two thirds the average weight. The few normal inbred young here considered are then equally as good as the young from normal non-relatives and necessarily superior to the alcoholic lines. Judging from the results of others, there is little doubt that a more extensive inbreeding might produce deleterious effects.

The last vertical column indicates the effects of inbreeding alcoholic animals and their descendants. This combination shows the poorest quality offspring found in the table. Here again the members of the larger litters are at a disadvantage when compared with those born in the small litters. The average number of young in a litter is 1.62, somewhat smaller than the litters produced by mating alcoholic non-relatives. Thirty-eight litters contained a single individual each, the same number of litters contained two individuals, while only seven litters consisted of three young, and these were the largest litters produced. The inbred alcoholic animals, therefore, have a tendency to produce a large proportion of small litters and this tendency aids in strengthening their offspring.

Of the 135 young resulting from these matings, only 36.29 per cent. of them survived; this is the poorest life record shown by any combination. Of those born only one in a litter, however, 76.31 per cent. survived, which is a record equal to the average of the control. Therefore, even in this very bad combination, alcoholic inbreeding, when only one young is produced at a litter by an animal ordinarily capable of producing two or more, this one young is so well nourished and accommodated that it is somatically vigorous. Yet on breeding such individuals it almost always happens that very inferior offspring result. The germ cells, at any rate, may possibly be stronger than those in the weaker individuals which occurred in litters of two or three. Only 26.31 per cent. of the animals born in litters of two were capable of sur-



FIG. 5. Ventral view of a normal guinea-pig brain killed three days after birth. The optic nerves, optic chiasma and optic tracts passing along the tuber cinereum are distinctly seen.

FIG. 6. Ventral view of the brain of an anophthalmic monster from alcoholic great-grandfathers. There are no optic nerves, chiasma or tracts; the only indication of optic parts being two small membranous processes about in the position of the optic chiasma.

viving. The mortality here happens to be about three times higher than among the single-litter individuals. And further, not one of the 21 specimens born in litters of three lived. Among the offspring from the alcoholic inbred lines, judging from the numbers now available, the difference between the vitality of individuals born in litters of one and those born in larger-size litters is most striking.

The space below is the reverse of the one just considered and gives the percentage of young dying in the different litters. Only 23.68 per cent. died from litters of one individual, while 73.68 per cent. died in the litters of two individuals, and every one, 100 per cent., of the animals born in litters of three died within three months and usually within a few days.

The proportion of deformed animals occurring in the different-size litters again emphasizes the same differences in quality. All together 14.81 per cent. of the 135 individuals were grossly deformed; this is the highest percentage of deformed animals occurring in the several combinations represented in the table.

Of those animals born one in a litter only 5.26 per cent. were deformed; of those born two in a litter 18.42 per cent., or more than three times as many, were deformed, and of those specimens born in litters of three 19.04 per cent., or about one in five of them, were grossly deformed.

The proportion of deformities, therefore, conforms to the mortality records, being very much higher in the larger litters, and not unusually high among the individuals born one in a litter as compared with the average percentage of deformities from alcoholic non-relatives. Therefore, the bodily quality of the offspring is not materially worse from alcoholic inbred animals than from matings of alcoholic non-relatives, provided only one individual is born in the litter. But when more than one individual occurs in the litter, the alcoholic inbred combination is the most disastrous for the vitality and form of the offspring of all the combinations considered.



The last space shows that 3.7 per cent. of these offspring were less than two thirds the normal size. This again compares unfavorably with the other combinations, and here also the individuals born one in a litter show a superiority over those born in litters of two.

From a consideration of this table it may be concluded that the vigor of a guinea pig varies inversely with the size of the litter in which the animal is produced, and this is equally true whether the animal is born from normal or alcoholic parentage. However, the differences between the mortality of animals born in litters of one, two or three from normal parentage are not nearly so great as comparable differences between the members of the small and large litters from alcoholic lineage. For example, the difference in mortality between normal animals in litters of one or two is about 1 per cent., or scarcely any; between these and the mortality of specimens born three in a litter there is a difference in mortality record of about 24 per cent., to the discredit of the larger litters.

The comparable differences in the alcoholic lines is ever so much greater. There is almost 14 per cent. higher mortality among individuals from litters of two than from litters of one, and actually about 43 per cent. higher mortality among animals from litters of three than from litters of one. The difference between the mortality percentages in the litters of one and the litters of two from alcoholic inbred animals is 50 per cent. In other words, the mortality is three times as high among individuals from litters of two as from litters of one in inbred alcoholics, while the normal individuals born in litters of two are equally as good as those in litters of one. The parents from the injured alcoholic lines are incapable of producing large litters of strong individuals. The sub-normal fetus fares pretty well alone in the uterus but is put at a great disadvantage by having to divide its uterine nourishment with brothers and sisters.

Another almost equally plausible explanation of this

striking difference in quality and vitality among the members of small and large litters might be given. It may be supposed that the growth capacity of the eggs maturing in the ovaries of normal and subnormal individuals depends somewhat upon the number of eggs maturing at any one time, or ovulation period. A normal animal may be capable of developing two entirely good eggs at an ovulation, or possibly three, whereas a weakened, less vigorous individual has ovaries incapable of producing more than one well-nourished or well-developed egg at any one time. Of course, it is understood that the small size of a mammalian egg would make it seem as though it required very little stored food from the ovary, yet that little must be of an extremely fine quality, since so much of the energy of early development is derived from the materials stored within the egg.

One point which might be interpreted to favor such an explanation is the fact that the small, weak young contained in the large litters do not recover and make their shortage good after birth, as might be expected if their inferior condition was simply due to a lack of nourishment available in the overcrowded uterine environment in which their late stages of development were passed. Lack of intra-ovarian nutrition would certainly produce a more lasting effect, since it occurs at an earlier stage than lack of uterine nutrition, though of course we do not pretend to deny that poor uterine nutrition would also leave its persisting mark.

When only one young was produced in a litter the average growth rate of such individuals during the first month after birth was 85.09 grams. Such specimens were not only largest at birth, but they grew fastest after birth. Animals born in litters of two increased 68.46 grams during the first month after birth, while those born three in a litter gained only an average of 63.6 grams during the same period. In other words, the last group only gained 75 per cent. of the amount gained by similar specimens which were fortunate enough to be developed alone in the ovary and in the uterus.

A second conclusion indicated by Table II is that inbreeding the defective alcoholic stock produces a quality of offspring decidedly inferior to that produced by the alcoholic lines when not inbred. This involves the internal factors of the germ cells. When a modified germ cell is united with a related one probably modified in a closely similar manner, a summation of the modification produces a more decidedly modified individual than would result from the combination of two non-related germ cells, even though they also be modified. In other words, as is shown in much of the data on heredity in higher animals, relatives probably respond to the treatment more nearly in the same way than do non-relatives, and therefore inbred defectives produce the most disastrous results obtainable.

THE RELATIVE CONDITIONS OF THE MALE AND FEMALE  
DESCENDANTS FROM PATERNAL AND FROM MATERNAL  
ALCOHOLIZED ANCESTORS

We may now consider the possibility of analyzing the relative influences of various alcoholized ancestors on their offspring of different sex and the descendants of such offspring. The problems may be stated thus: are the offspring from alcoholized males more or less degenerate or modified than those from alcoholized females, and is there a difference in the degree of degeneracy between the male and female offspring? Are the descendants from alcoholic grandparents on the father's side more or less defective than the descendants from alcoholic grandparents on the mother's side, and do alcoholized grandfathers and grandmothers show an equally strong tendency to stamp their grandchildren? Do the grandsons and granddaughters show relatively different conditions, depending upon whether they are descended from alcoholized grandfathers on the father's or the mother's side or from alcoholized grandmothers on the paternal or the maternal side?

Table III, which excludes all inbred animals, is a sum-

TABLE III

THE RELATIVE DEGENERATIVE INFLUENCE OF MALE AND FEMALE ALCOHOLIZED ANCESTORS ON THEIR MALE AND FEMALE DESCENDANTS. (ALL INBRED ANIMALS ARE EXCLUDED)

	Alcoholized Father		Alcoholized Mother				Alcoholized Grandfather on the Father's Side				Alcoholized Grandmother on the Mother's Side				Alcoholized Grandfather on the Mother's Side				Alcoholized Grandmother on the Mother's Side			
	Males with	Females with	Young of Un-known Sex with	Males with	Females with	Young of Un-known Sex with	Males with	Females with	Young of Un-known Sex with	Males with	Females with	Young of Un-known Sex with	Males with	Females with	Young of Un-known Sex with	Males with	Females with	Young of Un-known Sex with	Males with	Females with	Young of Un-known Sex with	
Total number..	44	43	70	37	23	38	36	38	26	34	32	48	37	33	36	23	18	25				
Lived over 3 months.....	37	33	0	34	22	0	26	23	0	23	19	0	25	23	0	18	14	0				
	84.1%	76.74%		91.89%	95.65%		72.22%	60.52%		67.64%	59.37%		67.56%	69.69%		78.25%	77.77%					
	44.58%			57.14%			49%			36.84%			45.28%			48.48%						
Died within 3 months.....	7	10	70	3	1	38	10	15	26	11	13	48	12	10	36	5	4	25				
	15.9%	23.25%	100%	8.1%	4.34%	100%	27.77%	39.47%	100%	32.35%	40.62%	100%	32.43%	30.30%	100%	21.74%	22.22%	100%				
	55.41%			42.85%			51.0%			63.15%			54.71%			51.51%						
Deformed.....	1	2	3	0	0	0	3	4	0	1	4	7	2	3	7	2	0	0				
	2.27%	4.65%	4.28%				8.33%	10.52%		2.64%	12.5%	14.58%	5.40%	9.03%	16.66%	8.69%						
	3.82%			0			7.0%			10.52%			10.37%			2.77%						

marized analysis of these questions. The male and female descendants from six different lines are tabulated. The table is not perfectly pure, but merely represents a mass result, since, for instance, in giving the young from alcoholized fathers some of these young had also alcoholized grandparents, etc. The same is true of the other lines. But the large majority of the figures are from unmixed matings, so that these mass results do have some real significance.

In the first vertical section is given the records of offspring from alcoholized fathers. Forty-four males, 43 females and 70 young of unknown sex are considered. Of the males 84 per cent. lived, and 76.7 per cent. of the females lived. These numbers are very high, since in the early part of the experiment only those young which survived were catalogued for sex. Therefore, all of the 70 young of unknown sex were animals which died at birth or soon after, and as the table shows more than half of the animals from alcoholized fathers died soon after birth.

The mortality among the male offspring from alcoholized fathers was 15.9 per cent., while among the female offspring it was considerably higher, being 23.25 per cent. The same difference in quality between the sexes is illustrated by the percentage of gross deformities. Only 2.27 per cent. of the males were deformed, while twice as great a proportion, or 4.65 per cent., of the female offspring from treated fathers were deformed. In all 3.82 per cent. of the offspring from alcoholized males were deformed and the female offspring were inferior in quality to the male.

The next section of the table presents similar data for the offspring from alcoholized mothers. There were 37 male, 23 female and 38 offspring of unknown sex. Again, the offspring in which the sex was ascertained during part of the experiment were only those that survived, therefore, their mortality record is very good, while all the animals of unknown sex were individuals that died shortly after birth. Yet the records of the males and fe-

males are based on exactly the same kind of data and are to be fully compared. Eight and one tenth per cent. of the males died, while only 4.34 per cent., proportionately about half as many, females died. Not one grossly deformed animal was found among the offspring of alcoholized females.

*Thus from the mortality records the sons of alcoholized mothers appear more affected than their daughters. And taken as a whole the records of the alcoholized mothers are superior in quality to those of the alcoholized fathers, thus indicating that the male germ cells are more injured by the treatment than the female germ cells.*

The third section shows the records of the male and female descendants from alcoholized grandfathers on the father's side. Here the mortality record of the males is much better than that of the females; 27.77 per cent. of the males died soon after birth, and 39.47 per cent., a very much higher proportion of the females, died.

The mortality of these animals from alcoholic grandparents seems much greater than that of animals from treated parents; this is due, however, to the fact that the sex of many more of these that died at birth was ascertained as they occurred later in the experiment when this point was being watched. The totals are the only figures in the horizontal mortality columns that are to be compared. The total mortality of descendants from alcoholic grandfathers on the father's side was 51 per cent., which is higher than the mortality of the offspring from alcoholized mothers, 42.85 per cent., but lower than the mortality of offspring from alcoholized fathers, which reached 55.41 per cent.

Among the ascertained male descendants from an alcoholized paternal grandfather 8.33 per cent. showed gross deformities, while 10.52 per cent. of the descendants ascertained to be female were deformed. Considering all the animals in this group, 7 per cent. were deformed, which is almost twice as great a proportion as occurred among the offspring of alcoholized fathers. *The deform-*

*ities in the  $F_2$  generation are more frequent than in the  $F_1$ .*

The fourth section shows the influence on the grandchildren of an alcoholized grandfather on the mother's side. This is the most injurious combination shown. Only 36.84 per cent. of the offspring survive. Of the male descendants of an alcoholized maternal grandfather 32.35 per cent. died soon after birth, while proportionally many more, or 40.62 per cent., of the female descendants died. In all a total of 63.15 per cent. of the descendants from alcoholized maternal grandfathers died, which is the highest mortality record obtained.

Among the grandchildren of alcoholized maternal grandfathers 10.52 per cent. were deformed, a very high proportion. But of the grandsons only 2.64 per cent. were deformed, while almost five times as many, or 12.5 per cent., of the granddaughters were grossly deformed. Thus *the females of the  $F_2$  generation from a treated maternal grandfather are poorer when considered from the standpoint of mortality record and bodily structure than the male  $F_2$ 's from the same source.*

The fifth line to be considered is that of an alcoholized grandmother on the father's side. The result of this treatment as shown by the grandchildren is very bad, but not quite so bad as from the alcoholized maternal grandfather just discussed.

From alcoholized paternal grandmothers the conditions of 37 grandsons and 33 granddaughters are to be compared. About the same survival record is shown by both sexes: 67.56 per cent. of the male grandchildren lived and 69.69 per cent. of the females lived. Of all the descendants from this combination, including those in which the sex was not determined, only 45.28 per cent. survived, giving a mortality record of 54.71 per cent., considerably better, by almost 10 per cent., than that of animals from an alcoholized maternal grandfather in the preceding section.

A large proportion of the animals from alcoholized paternal grandmothers were deformed, 10.37 per cent. However, only 5.4 per cent. of the grandsons were de-



formed, while many more, 9.03 per cent., of the granddaughters were deformed and among the young of undetermined sex 16.66 per cent. were deformed.

In the last section the records of descendants from alcoholized grandmothers on the mother's side are given. There were 23 males, 18 females, and 25 young which died with their sex unascertained. Forty-eight and forty-eight hundredths per cent. of the animals lived. The mortality among the males was 21.74 per cent., about the same as that of the females which was 22.22 per cent. The total mortality being 51.51 per cent. From this combination there occurred a low percentage of deformities, confined entirely to the grandsons. *So that 8.69 per cent. of the grandsons from alcoholized maternal grandmothers were deformed, while none of the granddaughters showed any gross structural abnormalities.*

TABLE IV

MORTALITY DURING THE FIRST THREE MONTHS OF THE DESCENDANTS OF KNOWN SEX FROM ALCOHOLIC ANIMALS (NOT INBRED)

Treated with Alcohol	Males			Females			All Together		
	Total Number	Died Early	Mortality	Total Number	Died Early	Mortality	Total Number	Died Early	Mortality
Father.....	44	7	15.90%	43	10	23.25%	87	17	19.54%
Mother.....	37	3	8.10%	23	1	4.34%	60	4	6.66%
Grandfather on father's side.	36	10	27.77%	38	15	39.47%	74	25	33.78%
Grandmother on father's side.	37	12	32.43%	33	10	30.30%	70	22	31.42%
Grandfather on mother's side...	34	11	32.35%	32	13	40.62%	66	24	36.36%
Grandmother on mother's side...	23	5	21.74%	18	4	22.22%	41	9	21.95%

Table IV presents in a more concise manner certain of the figures considered in the foregoing Table III. Only the mortality records of the male and female descendants from different sources and the total mortality of the several groups is shown by the table and thus a ready comparison of the conditions may be made. Among the offspring from alcoholic fathers 15.9 per cent. of the males died and 23.25 per cent. of the females. *The fe-*

*male are more injured than the male offspring of treated fathers. The next horizontal line shows that the offspring from treated mothers are far better than from treated fathers, having a much lower mortality. The male germ cell is more affected by the alcohol than the ovum, therefore treated fathers produce poorer offspring than treated mothers.*

The heterogeneous female descendants from an alcoholized paternal grandfather are more affected than the male, 39.47 per cent. mortality to 27.77 per cent.

The male and female descendants from an alcoholized paternal grandmother show about equal conditions, 32.43 per cent. male mortality to 30.3 per cent. female mortality.

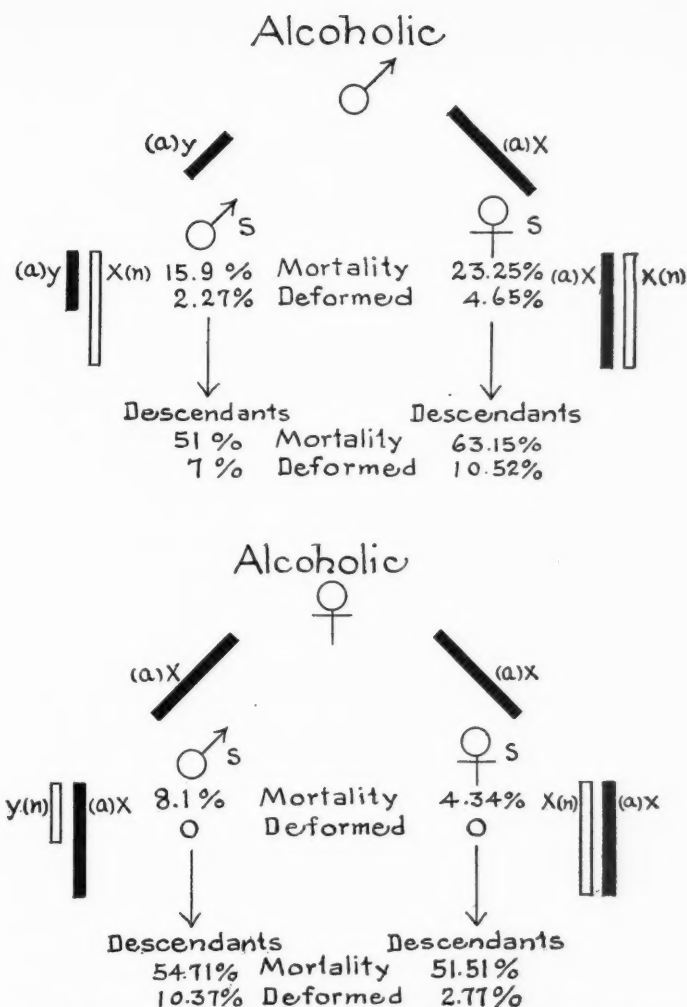
The heterogeneous female descendants are inferior to similar male descendants from an alcoholized maternal grandfather, 40.72 per cent. female mortality to 32.25 per cent. male mortality.

The male and female descendants are about equally strong from an alcoholized maternal grandmother, 21.74 per cent. male mortality to 22.22 per cent. female mortality.

Although explanations of the above differences between the ways in which the male and female guinea pigs are affected by the treatment, as well as explanations of the different records of the grandsons and granddaughters from alcoholization of different ones of the four grandparents, are difficult to give at the present stage of the experiment, a tentative explanation based on the composition of the chromosomal complex is certainly suggested.

#### GENERAL CONSIDERATIONS

In the case of the male guinea pig, according to the studies of Miss Stevens ('11), two kinds of spermatozoa are produced. The one has a large X chromosome, the female-producing spermatozoon, and the other contains a corresponding small Y chromosome, which from homology with other forms we may consider to be the male-producing. The two classes of spermatids are different



The diagram intended to show the difference in quality between the male and female offspring of alcoholized males and females, and indicating a possible explanation of these differences. The upper part of the diagram represents the heteromorphic chromosomes X and Y of the male passing to the male and female offspring. The larger injured X chromosome gives a larger proportion of deformities and a higher mortality to the females and their descendants. The smaller injured y chromosome gives to the males and their descendants a proportionately smaller amount of deformities and a lower mortality record.

The lower part of the diagram shows equal amounts of injured chromatin passing from the treated mother to her male and female offspring, the black X chromosomes. But these equal amounts of modified chromatin are combined with unequal amounts of normal chromatin from the heteromorphic spermatozoa, the unequal white X and Y chromosomes. The male offspring, therefore, have in their chromosomal constitution proportionally more modified and less normal chromatin than the female offspring which have equal amounts of normal and alcoholic. And the male offspring and their descendants are actually found to show a higher mortality and a greater proportion of deformities than the female offspring and their descendants.

morphologically and we may expect them to differ in their susceptibilities to the alcoholic treatment. One class may be more affected than the other. This might be due simply to the reason that one class of spermatozoa actually according to mass has more chromatin to be acted upon than the other. And this difference in mass of affected chromatin might be sufficient to give a difference in quality between the individuals arising from the two classes of spermatozoa.

At any rate, as the accompanying diagram indicates, there is a decided difference between the records of the male and female offspring from treated guinea pigs. The upper half of the accompanying diagram shows that the mortality is higher and the gross deformities more frequent among the female offspring sired by alcoholized male guinea pigs than among the male offspring. This difference we may venture to suppose is due to the fact that the female offspring actually receive more modified or injured chromatin from the alcoholic father than do the sons. The diagram is an attempt to represent this larger mass of injured chromatin, the large black X chromosome passing to the daughters, while the smaller black Y chromosome is received by the sons.

Another possible explanation might be that the two heteromorphic sex chromosomes, the X and Y, respond differently to the influence of the alcoholic treatment, the X being the more affected. Such an opinion has some basis, since these chromosomes in the later development of the two sexes seem to carry such a number of contrasting qualities according to the splendid evidence presented by Morgan and his associates. One may be permitted to assume on probability, at any rate, that the X and Y chromosomes are qualitatively different in their finer chemical constitutions, and this qualitative difference would necessitate a different response to the chemical treatment on the part of each of the two chromosomes.

There is also important evidence from the parthenogenetic groups, as, for example, the Phylloxerans and

Aphids (Morgan, '09), which might lead one to believe that the two classes of spermatids or finally spermatozoa are never quite equally active or vigorous. This difference may vary from apparent equality in most higher animals to cases such as the parthenogenetic Phylloxerans and Aphids, in which one class of spermatids are actually degenerate and non-functional.

In this connection an experiment performed with a quite different problem in view by Cole and Davis ('14) with alcoholized rabbits is suggestive. They found that when two male rabbits were mated with a female superfetation occurred in most cases so that part of the resulting litter of young were sired by one male and part by the other. The two males differed in their ability, so that one more often sired the majority of young of a given litter and in the total number of competition matings sired the greater number of young. When this male with the fertilizing advantage was treated for a short period of time, a month or more, with fumes of alcohol he was then affected in such a way that when mated in competition with the same male he normally had beaten he now failed to sire any young. Yet if mated singly or alone with a female he still had the power to beget offspring. The alcohol treatment had in some way lowered the power of his spermatozoa to fertilize an egg. Thus these spermatozoa could no longer fertilize an egg in the presence of the spermatozoa from a male which was originally less potent than they.

All of these data indicate differences in the behavior and reactions of the individual germ cells, and such differences probably account for the discrepancy existing between the conditions of the male and female offspring from an alcoholized father. Since this point has only recently been discovered in the experiments, we now have very few definite matings to test its meaning by back crosses with the normal. But a large number of heterogeneous matings have been made during the last few years and their gross results serve to verify the fact that

the difference in quality between male and female offspring is actual, although such matings furnish no definite analysis of the conditions.

In the first place, the upper half of the diagram shows that the mortality is higher and the defects more frequent among the female offspring of treated males than among their sons. The products of the heterogeneous matings in which these male and female offspring have taken part go to indicate that the first apparent difference in their records was a real difference. The mortality record of the mass descendants from the sons of alcoholized fathers is about 20 per cent. better than the mortality record from the descendants of the daughters of alcoholic fathers. And the proportion of deformities is 50 per cent. higher among the descendants of the daughters than among the descendants of the sons. These conditions of the descendants prove that the female offspring from the alcoholized males are actually worse than the male offspring in the following respects: their mortality record, the frequency of deformities, and the quality of young to which they give rise. The only plausible way to account for the origin of this difference is to assume that the female-producing spermatozoa were more modified by the treatment than the male-producing spermatozoa. Whether such an increased modification is due to the presence of a greater mass of chromatin to be injured in the one case than in the other or to a difference in response on the part of the two heteromorphic sex chromosomes it is impossible to state. The difference, however, is a fact!

The lower half of the diagram illustrates the different qualities of the male and female offspring from alcoholized mothers. Here each sex of the offspring in accordance with prevalent cytological views receives an equal amount of chromatin from the treated mother. And, moreover, as far as the treated mother is concerned similar chromosomal complexes are conveyed to both sexes of the offspring. The two classes of young should, therefore, show similar conditions, but such is not the case.

The mortality of the male offspring is higher than that of the female. This condition may probably be explained on the same principles we have employed above. The two sexes receive equal amounts of injured chromatin from their alcoholized mother, but this injured chromatin in the case of the female individuals is mixed with a larger amount of normal chromatin from the father than is the case with the male. The female combination of equal amounts of good and bad chromatin gives rise to a better product than the male combination of a larger amount of modified chromatin with a smaller amount of good. Therefore the records of the male offspring are inferior to those of the female offspring.

The female combination, ovum and spermatozoon with equal amounts of chromatin, good and bad, is proportionately less injured than the male combination, ovum with a larger amount of bad and spermatozoon with a smaller amount of normal chromatin. The diagram represents the black X chromosomes equal in size passing to the daughters and the sons to be combined with the large white normal X in the case of the daughters and with the small white normal Y in the case of the sons.

Again the descendants from heterogeneous matings of these males and females prove that there is an actual difference in quality. The descendants from the sons of alcoholic mothers show a slightly higher mortality and a much greater proportion of deformities than are found among the descendants of their daughters.

We believe that these results actually show a difference in response to the treatment on the part of the male- and female-producing spermatozoa. Such a difference logically follows the cytological differences in structure which Wilson and others have so clearly demonstrated during the past ten years. If this structural difference is of any significance, as it surely must be, then such physiological differences in behavior as are indicated in our results should sooner or later be found.

On such a basis as this the sex ratio in different classes

of animals may possibly be explained. A species such as man, which constantly seems to produce more males than females, may be said to form more active or vigorous male-producing spermatozoa. In the competition to fertilize the egg such spermatozoa win an advantage and in the sum total more males than females arise, the ratio depending upon the extent of the advantage the one class of spermatozoa has over the other.

We now have under way a number of matings which are designed to test these propositions in an analytical fashion. One of us (Papanicolaou, '15) is in possession of data giving reason to believe that a second explanation may be offered to account for the different conditions presented by the male and female offspring produced by alcoholized females. Such an explanation is based on the supposition that the female guinea pig as well as the male has a share in the determination of the sex ratio and may produce two kinds of ova. Such an explanation in its final analysis is extremely complex and unnecessary in the present discussion, though it will be presented in a future consideration of the regulation of the sex ratio in these animals.

Admitting, as is suggested above, that the two groups of spermatozoa differ in their response and resistance to the treatment, we may also admit that there are other normal differences in their vitality and behavior. These normal differences must also vary within certain limits. In one group of animals the female-producing spermatozoa may be more active and possess a higher degree of fertilizing power than the male-producing spermatozoa. Such a group would show a sex ratio below one hundred, there being more females than males produced. In other species of animals with a sex ratio of more than 100 the reverse condition obtains; the male-producing spermatozoa possess on an average a higher fertilizing power than the female-producing. But the advantage of the male-producing sperm may be slight and no doubt many individual males tend to form female-producing sperm



with a higher fertilizing power than the male-producing. Such individuals will more frequently beget female offspring. Slight differences in the physiological behavior of the two classes of spermatozoa would account for the sex ratios in all animals, and finally, as Morgan has shown, the extreme difference between the qualities of the two classes of spermatozoa leads to the degeneration of one entire class and the necessary production of only one sex from the fertilized eggs of these species. Such species must also be parthenogenetic in order to produce individuals of the other sex.

This discovery by Morgan suggests, as Wilson ('11) brings out in his review of the sex chromosome question, a plausible explanation of the sex ratios in different classes of animals. And we believe the evidence presented above lends further support to such an interpretation.

A rather old popular idea in attempting to explain the sex riddle may have some ground of fact from the standpoint of the variations in the differences of fertilizing power of the two classes of spermatozoa. It has often been claimed that one testis is male-producing and the other female-producing. Every one knows that this is untrue. Yet one testis may have a tendency to produce spermatozoa of the female class with a higher fertilizing power than the male sperm of this testis, and the other testis might have an opposite tendency, since the conditions of behavior often differ in two organs of a bilateral pair. An animal which has produced a large proportion of male offspring may after semi-castration produce almost all female offspring. A possible explanation for such an occurrence would be that the removed testis had produced more vigorous male sperm than female and the spermatozoa of this testis possessed the higher fertilizing power, while the remaining testis tended to produce more potent female sperm. On removing the one testis the other came into supremacy. In the same imaginary case, if the opposite testis had been removed, there would have

been no change in the tendency to produce offspring of a certain sex, since the remaining testis originally possessed an advantage.

Finally, then, from the above experiments there is no question that the material basis of the hereditary qualities has been injured, since alcoholized males have transmitted the injury to four generations during a period of almost five years. In other words, as stated above, chromatin injured five years ago is now living in the great-grandchildren of the individuals in which it was injured.

Bardeen with the X-ray and Oscar Hertwig with radium have induced similar injuries by directly treating the spermatozoa, but these cells were so greatly injured that only the immediate effect upon the developing embryo was shown. The present experiments, however, demonstrate the passage or transmission of the injured chromatin from generation to generation during a period of years. *The behavior of the carriers of heredity becomes pathological just as any other organ with a normal function may behave in an abnormal or pathological manner.*

Mammals are particularly adapted to the study of such features of heredity as this, on account of their typical structure and large, easily observed organs. The complexity of their structure and behavior further permit the possibility of slight modifications becoming visible through abnormal conditions of their nervous system, etc. Thus with such material as guinea pigs a few experiments of this kind may furnish certain clues to the processes of behavior of the chromosomes that less plastic and simpler forms might never present in such a manner as would be recognizable.

On the other hand, the small litters and comparatively slow breeding render these higher animals unsuitable for an exhaustive analysis of many of the intricate problems of normal heredity.

## SUMMARY AND CONCLUSIONS

In the foregoing pages we have considered the results of an experiment now in progress for more than five years which analyzes to some extent the influence on the offspring of alcoholizing either one or both parents and the manner of hereditary transmission of the induced effects to subsequent generations.

The experiments have demonstrated on two different stocks of normal guinea pigs that the parental germ cells may be so modified by chemical treatments that they are rendered incapable of giving rise to a perfectly normal offspring. This incapacity is probably due to modifications of the chromatin or carriers of the hereditary qualities within the germ cells, since the great-grandchildren, the  $F_3$  generation, from the treated animals are usually more decidedly affected and injured than the immediate offspring ( $F_1$ ) of the alcoholized animals.

This then becomes a study of the behavior of diseased or pathological chromatin in heredity. Chromatin rendered pathological more than four years ago is still living and has now been passed on to the  $F_3$  generation from the alcoholized great-grandparents. The  $F_3$  animals are almost without exception incapable of reproduction and are in many ways subnormal and degenerate.

Studies of abnormal heredity may possibly furnish a means of analyzing the normal methods of action by which the minute carriers of hereditary qualities contained within the fertilized egg are capable of causing complex developmental and structural changes to reoccur from generation to generation in so wonderfully consistent a manner. Just as the knowledge furnished by studies of experimentally modified embryonic development has supplied valuable data towards a clearer understanding of the normal processes and changes which occur in the developing embryo.

The treatment of adult guinea pigs by an inhalation method with daily doses of alcohol through several years produces little if any noticeable effect upon the organs

and tissues of the animal's body. The direct action of alcohol fumes tends to injure the respiratory mucosa and to render the cornea of the eye dull or opaque. These changes, however, do not inconvenience the animals in any perceptible way, and they remain strong and hardy and live as long and actively as the untreated guinea pigs. In spite of their healthy appearance the injurious influence of the alcohol inhalation is very decidedly shown by the quality of offspring to which the treated animals give rise. And the descendants of these offspring are even worse than the  $F_1$  generation when compared with the different generations of control animals produced under identical cage and food conditions.

The males seem to be more injured by the treatment than the females, taking as an index of injury the quality of their offspring and descendants. Stating it differently, the spermatocytes or spermatozoa are more sensitive to the changed chemical condition of the tissues than are the female germ cells.

There is a larger proportion of degenerate, paralytic and grossly deformed individuals descended from the alcoholized males than from the alcoholized females.

The records of 682 offspring produced by 571 matings of animals of various types have been tabulated to show the kinds of litters of young produced and their ability to survive. One hundred and sixty-four matings of alcoholized animals, in which either the father, mother, or both were alcoholic, gave 64, or almost 40 per cent., negative results or early abortions, while only 25 per cent. of the control matings failed to give full-term litters. Of the 100 full-term litters from alcoholic parents 18 per cent. contained stillborn young, and only 50 per cent. of all the matings resulted in living litters. Forty-six per cent. of the individuals in the litters of living young died very soon after birth. In contrast to this record 73 per cent. of the 90 control matings gave living litters and 84 per cent. of the young in these litters survived as normal, healthy animals.

The mating records of the descendants of the alcoholized guinea pigs, although they themselves were not treated with alcohol, compare in some respects even more unfavorably with the control records than does the above data from the directly alcoholized animals.

Of 194 matings of  $F_1$  animals in various combinations 55 have resulted in negative results or early abortions, 18 stillborn litters of 41 young occurred, and 17 per cent. of these stillborn young were deformed. One hundred and twenty-one living litters contained 199 young, but 94 of these died within a few days and almost 15 per cent. of them were deformed, while 105 survived and 7 of these showed eye deformities. Among 126 full-term control young of the same stock not one has been deformed.

The records of the matings of  $F_2$  animals are still worse, higher mortality and more pronounced deformities, while the few  $F_3$  individuals which have survived are generally weak and in many instances appear to be quite sterile even though paired with vigorous, prolific, normal mates.

The structural defects shown by the descendants of alcoholized animals seem to be confined chiefly to the central nervous system and special sense organs. Many of the young animals show gross tremors, paralysis agitans; the hind legs, fore legs or both legs of one side may be paralyzed (Plates I and II). Eye defects are very common, such as opaque cornea, opaque lens, various degrees of monophthalmicum asymmetricum, and finally several cases of complete anophthalmia have occurred, the entire eyeballs, optic nerves and optic chiasma being absent (Figs. 1 to 3 and Plate III).

The quality of individuals from the same parentage varies inversely with the size of the litters in which they are produced. Animals born one in a litter are rather strong, even though derived from very bad alcoholic lines. This difference between the members of small and large litters is also shown by the normal animals, but the difference in quality between members of large and small litters

is ever so much greater in the alcoholic lines. There is also some tendency on the part of the alcoholic animals to produce a greater proportion of small litters and this aids somewhat towards the perpetuation of their lines.

Inbreeding tends to emphasize the alcoholic effects. This is probably due to related animals responding to the treatment in closely similar ways on account of the similarity of their constitutions. Inbreeding, as such, may be harmful. But inbreeding added to the alcohol effects produces a much worse condition in the offspring than either inbreeding or alcoholism alone could do.

The data from alcoholized male lines indicate that the *female offspring from alcoholic males are less viable and more frequently deformed than the male offspring. And heterogeneous matings of such male and female offspring further emphasize the same inferiority on the part of the female offspring from treated males.* This is a very significant fact.

The fact that the offspring of one sex differ in quality from those of the opposite sex, and that the female offspring of an alcoholic male are inferior to his male offspring suggests at once a difference between the germ cells concerned in the production of the male and female young. Miss Stevens showed that the spermatocytes of the male guinea pig contained a heteromorphic pair of chromosomes and half of the spermatozoa would be expected to receive one member, the X chromosome, of the heteromorphic pair and one half of the spermatozoa the other member, the Y chromosome, of the heteromorphic pair. We now have two possibilities in explanation of the above facts. In the first place, it may be assumed that the alcohol acts similarly on all of the chromatin to injure it. Thus a mass action would cause the spermatozoa carrying the larger member of the heteromorphic pair to deliver more injured chromatin and the other spermatozoa with a less total amount of injured chromatin would deliver less when they fertilize eggs containing equal amounts of normal chromatin. The fertilized

egg giving rise to the female, therefore, contains a greater proportional amount of alcoholic chromatin to normal chromatin than does the egg giving rise to the male. And so the female product is actually more injured than the male.

A second possible explanation of these conditions may be that the X and Y chromosomes themselves respond differently to the treatment, the X being the more sensitive of the two. But in either case the two classes of spermatozoa certainly seem to respond differently to the treatment and this shows a physiological difference in behavior to correspond with the well-known morphological differences so often found between the two groups of spermatids of many animal species.

The data from alcoholic female lines indicates that *the male offspring from alcoholic females are inferior in quality to the female offspring. And heterogeneous matings of such male and female offspring further prove the inferiority on the part of the male offspring from treated mothers.* This is also significant. How can it be put in accord with the above chromosomal explanations for the difference in quality between the female and male young of alcoholized fathers?

If we admit that all of the eggs arising from an alcoholized female guinea pig are homomorphic and contain groups of chromosomes equal in mass, it follows that her male and female offspring receive the same amount of injured chromatin and should be affected by such chromatin to equal degrees. But this is only part of the case, the injured female chromatin is combined with normal chromatin from the normal father when the eggs are fertilized and here the difference arises. The female offspring receives from the normal father a larger amount of normal chromatin than do the male offspring. So that the female arises from an egg in which equal amounts of good and injured chromatin are present, while the male offspring arises from an egg in which a larger amount of injured chromatin is united with a smaller amount of



normal. Therefore, proportionally, the male offspring from treated mothers have more injured chromatin in their entire bodily make up than do the female offspring, and are comparatively in a more abnormal condition.

Another explanation of these differences between the male and female offspring of alcoholized females could be based on the possibility of the female being heterozygous for sex. This involves a very complex discussion, but one for which there is some ground on the basis of the regulation of the sex ratio in these animals.

Finally, then, the experiments show the hereditary transmission through several generations of conditions resulting from an artificially induced change in the germ cells of one generation. And they furnish data of importance bearing upon the pathological behavior of the carriers of heredity as well as the differences in behavior between the two types of germ cells produced by an animal carrying heteromorphic chromosomes.

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## SHORTER ARTICLES AND DISCUSSION

### VARIABILITY UNDER INBREEDING AND CROSS-BREEDING

AN unusually thoughtful and suggestive discussion of evolutionary problems is contained in Professor Walton's paper on "variability and amphimixis" published in the November, 1915, number of this journal. But the paper is in some danger of neglect because the conclusions reached are apparently so revolutionary that to a casual reader they may seem freakish. Yet it will be seen by one who reads the paper more carefully that the radical character of its conclusions is due in part to the fact that certain familiar ideas are here viewed at a new angle. Nevertheless the new point of view has, it seems to me, to some extent, caused the author loss of perspective in relation to some of the phenomena which he discusses, for which reason further consideration of them may be profitable.

The occasion of Walton's discussion was a biometric study which he made of two sorts of zygospores produced by *Spirogyra inflata*, one sort produced by union of cells in the same filament (called by him "close fertilization"), the other by the union of cells in different filaments (called "cross fertilization"). Zygospores of the former sort ("close fertilized") were found to be on the average larger and more variable than those of the latter sort, *contrary to the prevailing idea that cross fertilization leads to increased variability*. It may however be questioned whether Walton's material is such as to throw new light on this question, for it is by no means certain that cells of *Spirogyra* which unite in lateral conjugation are the exact equivalents morphologically and physiologically of those which unite in scalariform conjugation. It is conceivable that zygospores formed in lateral conjugation may be larger and more variable because the cells which gave rise to them were as a group larger and more variable. It is conceivable that cells which resort to scalariform conjugation are not such as can satisfy their physiological demands for conjugation by uniting with a sister-cell in the same filament. For it is known that in many plants sexual union occurs only as a last resort, when conditions

are unfavorable for further continuance of the organism either by vegetative reproduction or even by parthenogenesis.<sup>1</sup> But whether or not Walton's own observations are considered pertinent, the question which it leads him to consider is one of profound evolutionary significance—does cross fertilization (as compared with close fertilization) tend to produce greater or less variability.

In parts of his discussion Walton fails to keep clearly in view distinctions, which he nevertheless recognizes, between the respective variabilities of  $F_1$ ,  $F_2$  and mixed populations. The fact has been known since the days of the early plant hybridizers, and is expressed clearly in one of Focke's laws of hybridization that the first generation ( $F_1$ ) offspring of a hybrid cross are not, as a rule, more variable than the more variable parent race. In other words the generalization which Walton attacks, that crossing produces variability, is not commonly, if at all, held by biologists to apply to  $F_1$  populations but only to the conditions obtaining in subsequent generations. But Walton's own observations are made exclusively upon  $F_1$  zygotes. Supposing his two classes of zygotes to be morphological and physiological equivalents of each other (which, however, may reasonably be questioned) there was no ground for expecting one sort to be more variable than the other, so far as existing knowledge of the effects of inbreeding and cross-breeding is concerned.

Walton cites two experimental investigations, in support of his own observations on *Spirogyra*, to show that close fertilization produces greater variability than cross fertilization, viz., that of Jennings on *Paramecium* and that of Barrows and myself on *Drosophila*. But neither of these investigations deals with the same sort of cases as Walton's. Jennings is comparing the variability of conjugants with that of non-conjugants. This is a case where sexual is contrasted with asexual reproduction and is in no way comparable with a case in which the effects of cross and close fertilization are compared with each other. I quite agree with Walton's conclusion that the results are statistically considered far from conclusive, and would add that they are quite aside from the question which Walton is considering. Barrow's comparisons were made between *single lines* of *Drosophila* inbred (brother with sister) for from 30 to 61 generations and a culture derived from two original pairs of

<sup>1</sup> See Coulter, 1914, "The Evolution of Sex in Plants."

*Drosophila* the descendants of each pair being allowed to interbreed freely. As to the results we said (p. 776) :

These experiments show no appreciable effect of inbreeding (on variability). In every case the brood reared under the best and the most uniform conditions has the highest average number of teeth (in the sex-comb), irrespective of whether or not inbred. The same may be said of variation in size. Inbreeding has diminished neither the average size nor the variability in size.

Walton considers these conclusions justified by our statistical constants in the case of the sex-comb, but believes that a significant difference is observed in length of tibia, which we found to be both greater and less variable in the culture not inbred. He criticizes our failure to calculate coefficients of variation for tibia length (as we had done for sex-comb) and upon calculating such coefficients finds the greater variability of the inbred lots significant. But the same difference in variability was indicated by the standard deviations (which we gave) and the calculation of the coefficient of variation adds nothing to the force of the demonstration. We considered then and still consider the differences observed sufficiently accounted for by external conditions, *i. e.*, we considered them purely phenotypic. We showed that length of tibia is greatest and its variability least when food and temperature conditions are best. The difference between two inbred races (*M* and *N*) inbred practically the same number of generations (*viz.*, 31 and 30, respectively) but treated very differently as regards food, was found to be several times greater than the difference between the inbred culture *M* and the not-inbred culture *X*. Hence it is not probable that the inbreeding had anything to do with the differences found in variability.

It is difficult to understand how on any theory of heredity inbreeding could be expected to *increase variability* within a single inbred line, such as one of our inbred cultures of *Drosophila*. On a Mendelian theory it would be expected that inbreeding, brother with sister, for a large number of generations (61 in our *A* series) would result in the production of a number of homozygous lines, each of which by itself would be *entirely devoid* of variability, except that due to environmental agencies. If all the possible derived lines descended from a pair under inbreeding were combined into one mass of material, it would seem probable, on a Mendelian theory, that if any genotypic varia-

tions had occurred, this material would show greater variability than the ancestral race before inbreeding began. This, I take it, is the point which Walton has in mind when he asserts that inbreeding has a tendency to increase variability. But this is very different from the condition to be expected in any *single line* considered separately, as in one of our inbred lines of *Drosophila*. Such a line should be *less variable* than the population from which it arose, provided that population contained any genotypic variations whatever!

The question is decidedly worthy of consideration, which Walton's paper suggests, is evolution more rapid in a self-fertilizing or habitually close fertilizing population on one hand or in a habitually cross fertilizing population on the other hand. The importance of the question is not lessened by the fact that Walton has brought into the discussion material wholly irrelevant, including his own observations on the zygospores of *Spirogyra* and the observations of Jennings on *Paramecium* and Barrows on *Drosophila*. But the work of Hayes on the variability of pure races of tobacco and of their hybrids, which Walton cites, does bear directly on this question. By combining the observations on the parent races into one mass of data and treating this statistically, Walton has shown that self-fertilizing lines *mixed together* would form a population more variable as regards number of leaves and height of plant than the population produced by cross-breeding of these same lines. Hayes's observations verify Focke's law already cited, that the variability of  $F_1$  does not exceed that of the more variable parent race, but that  $F_2$  shows increased variability. Theoretically  $F_2$  should show the *maximum* variability. Walton's figures indicate clearly that this maximum variability under cross-breeding is *less* than the variability of a mixture of the two inbred races and consequently that continuous self fertilization within a mixed population will produce a more variable population than will result from continuous cross fertilization. This is an important generalization which demonstrably will hold good in all cases in which "intermediate" or "blending" inheritance occurs. It would not hold good for cases in which completely dominant and quantitatively invariable Mendelian factors are concerned, but it is doubtful whether such cases occur, as I have elsewhere tried to show. It is the great variability of self fertilizing populations and the stability of variations arising under self fertilization (since no variations will be "swamped by cross-

ing" in a self fertilizing population) that allow of the formation and perpetuation of "little species," side by side and yet quite distinct, within highly variable taxonomic species such as the dandelion. These same characteristics of self fertilizing populations furnish much of the material which plant breeders use. Following Vilmorin, they find it necessary only to *isolate* and propagate by themselves the variations which spontaneously arise. The task of the breeder who is dealing with a continually cross-breeding organism is much more complex. He often finds it necessary *first to inbreed* his stock, in order to learn what potential variations it contains, or, if one prefers so to express it, in order to induce variability, though this form of statement is not strictly accurate. Such inbreeding of a naturally cross-breeding organism often causes temporary loss of vigor, as notably in the case of maize, and frequently in domestic animals. But when the desired variations have been isolated, vigor can usually be recovered by increasing the stock to such an extent that matings become possible within the race and yet not involving union of closely related individuals.

Notwithstanding the utility of inbreeding in securing variations, there are important sources of variability which are found in cross-breeding alone. Supposing that under inbreeding variation has already occurred in different directions and the original condition has been wholly lost, it is often possible to recover it again by crossing. This is the familiar phenomenon of reversion upon crossing. It is also possible by crossing to combine in one race variations which have occurred separately in different races, a thing which would be impossible under continuous inbreeding. But a certain amount of inbreeding must usually in such cases follow up the cross-breeding in order to isolate and make secure the combinations desired.

It is not wise, therefore, unduly to exalt either inbreeding or cross-breeding as evolutionary processes or tools of the breeder. Each has its utility at the proper time and place. They are like pick and shovel, each supplementing the work of the other.

The question is worth considering in this connection—what effect will inbreeding and cross-breeding respectively have on the variability of single characters. This is a question to which I have given considerable attention for several years and the answer to it is, I think, becoming clear. A single character which Mendelizes has its variability increased by crossing. Some explain this as due to actual modification of the unit character

through crossing, others as due to the introduction of modifying factors by means of the cross. Whichever view is adopted, the fact is perfectly clear that modification of single Mendelizing characters occurs in cross-breeding. Under continuous inbreeding we should expect that single Mendelizing characters (within single lines but not within an entire inbred population) would attain a condition as devoid of variability as it is possible for them to attain and observation confirms this expectation.

As regards characters which "blend" in heredity, these are not inherited as single characters; they do not Mendelize in the ordinary acceptation of the term. The characters of the respective parent races *disappear* in the cross, being replaced by a common intermediate condition or blend. This blend persists into the  $F_2$  and later generations but with a certain amount of variability which is at a maximum in  $F_2$  and beyond that point tends to disappear in the absence of any special selection. It points to imperfection of the blending process or, in the view of those who prefer a Mendelian terminology for such cases, it points to *plurality* of factors determining the character. All the cases with which Walton has dealt in the paper under review are cases of *blending* inheritance and as regards them it is true, as already indicated, that continuous inbreeding tends to the production of a *more varied population* (but not of more variable separate lines) whereas cross-breeding tends to produce a *less variable population* (devoid of differences between families) but nevertheless a population more variable than the single lines of a self fertilizing or constantly inbred population.

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## NOTES AND LITERATURE

### MIMICRY IN BUTTERFLIES

AMERICAN biologists have been somewhat in a quandary of late as to what to believe and to teach about "mimicry" in insects. The consideration of chance resemblances in animate and inanimate things in which mimicry in the strict sense could not possibly exist, and the widespread skepticism of natural selection as an effective, creative agency in evolution have made many of us inclined to bury mimicry in the same grave with teleology, prenatal influences, the inheritance of acquired somatic characters, and sexual selection. Meanwhile, the Oxford school of zoologists, under Professor Poulton's leadership and the inspiration of an orthodox faith in the potency of natural selection, have continued to accumulate a rich array of newly discovered models and mimics among African butterflies.

Many of these and other cases of mimicry are described in the opening chapters of Professor R. C. Punnett's interesting book and admirably portrayed in the sixteen plates, twelve of which are in colors. With a remarkably clear and convincing style that has become familiar to us through his popular little book on Mendelism Punnett here recounts the history of the theories of Bates and Müller, mentions some of the morphological features upon which real affinities among butterflies depend, and describes in some detail examples of mimicry from various parts of the world.

Of particular interest to us in the United States is his brief discussion of the supposed mimicry of *Papilio philenor* by *P. troilus*, by the black southern variety, usually called *glaucus*, of the female of our common *turnus*,<sup>2</sup> and by a third species, *P. asterius* (usually known by us as *P. polyxenes*, or *P. asterias*). The northward extension of the range of *troilus* into Northwest Canada, far beyond that of the supposed model *philenor*, is thought to weaken this as a case of mimicry, and the author concludes that

<sup>1</sup> "Mimicry in Butterflies," by R. C. Punnett, Cambridge Univ. Press, 1915, 8vo., pp. 159, 16 plates.

<sup>2</sup> Punnett transposes these names, following Poulton (*vide Annals Entom. Soc. America*, Vol. 2, 1909, p. 225), who adopts Rothschild and Jordan's revision.



On the whole it seems at present doubtful whether any relation of a mimetic nature exists between *P. philenor* and these three species of *Papilio*.

The blue female of the southern fritillary, *Argynnis diana*, and our "red-spotted purple," *Limenitis (Basilarchia) astyanax*, which Professor Poulton has conceived also to be mimics of *P. philenor*, are likewise regarded as "very problematically mimetic." The striking resemblance of our "viceroy," *L. (B.) archippus*, for the "monarch," *Danaïs (Anosia) plexippus*, is mentioned, though no allusion is made to Abbott's biometrical study of 87 specimens of the supposed ancestral type, *L. (B.) arthemis*, from which the mimic, *archippus*, is thought to have arisen. Abbott,\* by the way, found that the color markings involved in the Poulton hypothesis of gradual change by natural selection (*e. g.*, reddish spots) are much less variable than the blues and other colors not considered in that theory, the color pattern of *arthemis* showing no tendency to break up or to shift in the direction of the *Anosia* type.

Punnett next examines critically Wallace's well-known laws or conditions of mimicry, discusses the evolution of a Ceylonese "mimicry ring" (a group of five superficially similar butterflies), describes the case of *Papilio polytes*, the trimorphic "mimetic" and "non-mimetic" females of which are genetically separated from one another by two Mendelian factors, considers the enemies of butterflies, and, finally, the relation of seasonal and local variation to mimicry. He arrives at the general conclusion that there are two prominent difficulties in "accepting the mimicry theory as an explanation of the remarkable resemblances which are often found between butterflies belonging to distinct groups," viz., "the difficulty of finding the agent that shall exercise the appropriate powers of discrimination, and the difficulty of fitting in the theoretical process involving the incessant accumulations of minute variations with what is at present known of the facts of heredity."<sup>3</sup> In view of these difficulties, taking his cue from genetics, he suggests that

Each group of Lepidoptera contains, spread out among its various members, a number of hereditary factors for the determination of color pattern. . . . Some factors may be common to two or more groups, in which case some of the permutations of the factors would be similar in the groups and would result in identical or nearly identical pattern.<sup>4</sup>

\* Washington Univ. Studies, Pt. 1, No. 2, 1914.

<sup>3</sup> P. 139.

<sup>4</sup> Pp. 145, 146.

Thus, referring by way of illustration to the somewhat analogous case of the coat colors of rodents, he says:

In certain features the rabbit might be said to "mimic" the mouse, in other features the guinea-pig.

It is a significant fact in this connection that the various models "mimicked" by the different species of a polymorphic species are almost always closely related, and hence may be expected to exhibit color patterns based on different combinations of identical factors.

In criticism of Wallace's laws of mimicry, Punnett points out the fact that although the mimic and model usually occur in the same locality this is not always the case, the cooperation of migratory birds being invoked to explain the exceptions.

Regarding the defenselessness of mimics as compared with models, it is noted that the "mimic" is often a swifter flyer, and hence better prepared for defense than the model.

Exceptions are given to the rule that the models are more numerous than the mimics, and that the mimics differ from the most of their nearest allies. The Pierid genus *Dismorphia*, for example, includes prominent South American mimics which differ strikingly from the "whites" of the Temperate Zone but, unfortunately for the theory of mimicry, only about a dozen of the seventy-five described species are white, the rest presenting a "wonderful diversity of color and pattern." Among them are species clearly non-mimetic as regards color, which by simple substitution of one color for another in the spots would be transformed into a "mimetic" species.<sup>5</sup>

The author concludes that

It is on the whole unusual to find cases where a single species departs widely from the pattern scheme of the other members of the genus and at the same time resembles an unrelated species.

Two of the best examples are our American "viceroy" and the pierid *Pareronia*. "Mimicry tends," he adds, "to run in certain groups" and "in many cases at any rate little meaning can be attached to the statement that the imitators differ from the bulk of their allies."

<sup>5</sup> The reviewer recently observed in Porto Rico a case bearing upon this point, in *Leptalis* (*Dismorphia*) *spio*, which closely resembles in color and general shape the very common *Heliconius charitonius*. A color variety of the former, however, is found in certain localities on the island, in which orange replaces yellow in the color pattern, rendering the resemblance to the Heliconian less apparent. A simple mutation of orange into yellow would make this an excellent example of "mimicry."

In the chapter entitled "Mimicry Rings" the author considers the difficulty of explaining the protective value of the minute initial variations in the direction of a model. As an illustration, a group of five superficially similar butterflies in Ceylon is described. This "mimicry ring" includes two hypothetically distasteful Danaines (*D. chrysippus* and *D. plexippus*) and the females of three very unlike males (*Hypolimnys misippus*, *Elymnias undularis*, and *Argynnis hyperbius*). The coloration of one of these males (*E. undularis*) is a deep purple brown, like that of "satyrs" generally. If this represents the original type from which the gay orange and black pattern of the female has been derived, how has the change come about? Slight initial variations of the Satyr in the direction of the orange Danaine could not possibly be mistaken by birds for the model. The absurdity is pointed out of assuming, on the other hand, that the Danaine was originally like the male Satyr, and acquired its warning coloration *pari passu* with the mimic, for the Danaine model can hardly have been originally like all of the three very diversely colored males of the mimicking females. Mutation in each of the three types, however, may have produced females so similar to the Danaine as to be mistakable for it, and if natural selection indeed operates in this case, it may act in "putting on the finishing touches," or in preventing regression.

In the two following chapters the author discusses the resemblance of two of the three varieties of female *Papilio polytes* to the two "poison-eating" Papilios of India and Ceylon, *P. aristolochia* and *P. hector*. As is well-known, Punnett<sup>6</sup> has himself studied in Ceylon the behavior of these species, and Freyer<sup>7</sup> has continued the work, making extensive breeding experiments on the polymorphic "mimic."

A study of the geographical distribution in this case shows a general correspondence between the range of each mimic and its model, but notable differences are discovered.<sup>8</sup> Regarding the value of the resemblance between mimic and model, Punnett had no difficulty in distinguishing between model and mimic on the wing, even at a distance of forty to fifty yards, while near at hand the brilliant scarlet of both models, which covers the body and is conspicuous in spots upon the wings, is seen to be very different from the softer red found upon the wings (not

<sup>6</sup> "Spolia Zeylanica," Vol. 7, Part 25, 1910.

<sup>7</sup> *Phil. Trans. Roy. Soc.*, London, Vol. 204, 1913.

<sup>8</sup> *Vide*: Lutz, *AMERICAN NATURALIST*, Vol. 45, p. 190, 1911.

upon the bodies) of the mimics. Dried specimens of models and mimics are likely to be confused, but not the living butterflies.

Freyer's breeding experiments bring out the fact that a simple Mendelian relation exists between the three varieties of female in *P. polytes*, the males of which, though phenotypically alike, correspond genotypically to the three kinds of female. Of these three the one resembling the male ("non-mimetic") is recessive to the mimetic forms, lacking a factor,  $X$ , possessed either in simplex or duplex condition by the "mimetic" females. The male likewise is latently either  $xx$ ,  $XX$ , or  $Xx$ , as the case may be, but retains a uniform appearance in all cases owing to the presence of an inhibitor factor for which he is heterozygous ( $Ii$ ), the female being recessive ( $ii$ ). The male, unlike other Lepidoptera, so far as they have been investigated, is also supposed to be heterozygous for a sex factor which we may for brevity call  $M$ , responsible for maleness, with which the inhibitor factor is completely coupled, so that the male-producing sperms ( $MI$ ) always contain the inhibitor factor, the female-producing always lack it ( $mi$ ).

The two mimetic varieties of female are differentiated from each other by the presence or absence of another factor,  $Y$ , which acts merely as a modifier of the factor  $X$  when that is present, and transforms the aristolochiæ-like female ( $XXyy$  or  $Xxyy$ ) into the hector-like ( $XXYY$ ,  $XXYy$ ,  $XxYY$ , or  $XxYy$ ). This color modifier, responsible for intensifying and extending the red markings, is supposed to occur in either homozygous or heterozygous condition, or to be absent (recessive) in the male-like form of female and also in each biotype of the male, though when present without  $X$ , or in the presence of  $I$ , it has no visible effect. Thus there are 9 biotypes of males and 3 of male-like females, all phenotypically alike. Referring to Poulton's theory of the gradual evolution by natural selection of the male-like type of female into the aristolochiæ-like, and subsequently into the hector-like, Punnett argues that crossing the hector-like (double dominant) with the male-like (double recessive) germ plasms and inbreeding should show the hypothetical intermediates postulated by Poulton, but no such intermediates have appeared in the breeding experiments.

Freyer's random sampling of the population of *polytes* gave 49 of the two mimetic females to 40 of the male-like coloration, or roughly 5 dominants to 4 recessives, a proportion indicating

stable equilibrium between the mimetic and non-mimetic varieties. Scanty historical data tend to show that the mimics were as common fifty years ago, and probably a century and a half, as to-day, so the author concludes "that in respect of mimetic resemblances natural selection does not exist for *P. polytes* in Ceylon," or at least there is "no effect appreciable to the necessarily rough method of estimation employed."

The author next considers the evidence that the enemies of butterflies could have played the part assigned to them by the advocates of the mimicry theory. Predaceous insects evidently pay no attention to warning colors; certain lizards devour butterflies freely, but do not exercise any discrimination in the species which they attack. Hence neither insects nor lizards can be supposed to play any part in establishing a mimetic resemblance. Birds destroy butterflies in considerable numbers, but

Some of the most destructive appear to exercise no choice in the species of butterfly attacked. They simply take what comes first and is easiest to catch.

Monkeys and baboons often eat butterflies. They show strong likes or dislikes for certain species. The monkey may be regarded as "the ideal enemy for which advocates of the mimicry theory have been searching—if only it could fly." The conclusion is reached that

even a slight persecution directed with adequate discrimination will in time bring about a marked result where the mimetic likeness is already in existence. It is not impossible therefore that the establishing of such a likeness may often be due more to the discrimination of the monkey than to the mobility of the bird.

In the final chapter on "Mimicry and Variation" the author describes Carpenter's observations on the polymorphic mimic *Pseudacraea eurytus*, the four forms of which show an extraordinary resemblance to acraeine "models" of the genus *Planema*. These butterflies inhabit the shores of Victoria Nyanza in Central Africa where the models are very abundant, the polymorphic mimics less common but still numerous, and intermediates between the different types of mimic rare, but not unknown. On Bugalla Island in the lake, on the contrary, the mimetic *Pseudacraeas* are very abundant, and their respective *Planema* models relatively rare. Here intermediates between the varieties of the polymorphic mimic occur in proportionately larger numbers than on the mainland, owing as Dr. Carpenter believes to the

cessation of natural selection in the absence of sufficient models to familiarize the hypothetical enemy with the several warning color patterns of the models. On the mainland, however, any of the aberrant intermediates that might be produced by interbreeding of the different varieties of the polymorphic species would meet an enemy having constant experience with the warning colors of the different models, and tend to be eliminated. The enemy, in other words, would avoid the perfect mimics, while aberrant individuals suggesting two models at once presumably would be attacked and eaten. This interesting case deserves thorough investigation.

The author makes a *faux pas* when he says regarding seasonal variations in butterflies, due to "changes in the conditions of later larval and earlier pupal life":

In no case are they known to be inherited, and in no case consequently could variation of this nature play any part in evolutionary change.

In the example cited (*Araschnia levana-prorsa*), which presents two color patterns alternately through the year, it is obvious that both patterns are inherited. The environment indeed decides which shall appear, but the hereditary basis common to both seasonal types is no less real than that of any butterfly of seasonally uniform pattern. Although the seasonal color patterns of *A. levana* and *prorsa* apparently can not behave as Mendelian allelomorphs to one another as do the color patterns of other non-seasonal polymorphic insects, they are by no means outside the pale of Mendelian heredity. It is not too much to expect that future studies will disclose colors or color patterns allelomorphic to *A. levana-prorsa*'s shifting coloration. The reviewer would not, with Professor Punnett, rule seasonal variation entirely out of court as possible stages in "the development of a mimetic likeness" or, rather, in the evolution of the remarkable likenesses, alleged to be mimetic, which this book brings so well to our attention.

The author is so strongly influenced by the idea that minute variations are fluctuations always controlled by the environment rather than by the internal conditions that result in heredity that he treads upon uncertain ground in discussing an example of local variation cited by Poulton.<sup>9</sup> A small white spot on the wing of *Danaus chrysippus* varies in size locally from a conspicuous marking, in China, to a faint dot tending to dis-

<sup>9</sup> Bedrock, 1913, p. 300. Cited by Punnett.

appear, in Africa. Punnett suggests that the details in pattern may be in slight measure affected by the plant species on which the caterpillars have fed, thereby producing local races. Transportation of a local race to a region inhabited by another distinct local race "would help us in deciding whether any variation by which it is characterized had a definite hereditary basis or was merely a fluctuation dependent upon something in the conditions under which it had grown up." We may well ask: are these two propositions mutually exclusive? May not a detail of color pattern to a certain degree at certain times be subject to environmental influences and at the same time may not its variations have a "definite hereditary basis?" The reviewer has had so much experience in observing the transmission in *Colias philodice* and *C. eurytheme* of spots comparable to that mentioned in *D. chrysippus* that he is convinced that a definite hereditary basis (consisting presumably of multiple factors) underlies every fluctuating detail of color pattern. By artificial selection from inbred stock, using uniform food plants, and exposing the caterpillars and pupæ to similar conditions, the breeder of butterflies may decrease even to elimination or increase within certain limits a detail of color pattern like that mentioned. The champions of the theory of mimicry are entitled to this crumb of comfort. "For if it can be supposed," remarks the author, "that small differences of this nature are always transmitted, it becomes less difficult to imagine that a mimetic resemblance has been brought about by a long series of very small steps."

Yet the facts which the mimicry theory seeks to explain clamor for explanation. Punnett sets forth at the end some that are most insistent.

Certain color schemes are characteristic of distinct geographical regions in South America, where they may occur in species belonging to very different genera and families.

In Central America a pattern occurs that is common to several Heliconines, Ithomiines, Nymphalines of two or more genera, and Pierids; in eastern Brazil another pattern in which "all the various genera which figure in the last group are again represented." On the upper Amazon a still different pattern is common to the same group of genera from that just mentioned, only two notable genera being absent. Finally in Ecuador, Peru and Bolivia a widely different pattern occurs in a group lacking



a Pierid or Danaid but containing in exchange "a *Papilio*, an *Acræa*, and two species of the Satyrid genus *Pedalodes*."

Assuming that one of these patterns must have been the most primitive, he asks why a distasteful genus should change from one efficient warning pattern to another quite distinct one. Though the premise is not necessarily true nor even probable, yet if the ancestral pattern were a generalized type of any "warning" character whatever, the question would still be pertinent.

The author suggests that a newly acquired color scheme, like one of these, may be "associated with a certain physiological constitution which places butterflies possessing it at an advantage as compared with the rest," just as the melanic variety of peppered moth that is ousting the typical form in Britain and on the Continent may have associated with its deep pigmentation a greater hardiness. This, however, goes but a short way toward the explanation of the extraordinary local associations of unlike South American butterflies showing similar coloration. This is a live question that challenges the attention of any student of evolution who has opportunity to undertake experimental work in the tropics of South America.

So if we must sooner or later consign Mimicry to its last resting place, with its less infirm but already moribund parent, Warning Coloration, let us do so filled with gratitude for the pioneer work accomplished by its champions in opening up promising fields of investigation, where we or our descendants may hope to discover new factors in evolution or to gain a deeper insight into those now only dimly understood. Thanks are meanwhile due to the author of this attractive volume for his keen diagnosis of the present condition of the mimicry theory and for his admirable description of the phenomena which it has attempted to explain.

JOHN H. GEROULD



